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Published in:
Proceedings

Publication date:
2011

Document Version
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

Citation (APA):
Sárossy, Z., Egsgaard, H., & Plackett, D. (2011). Isolation and chemical characterization of hemicelluloses from rye bran. In *Proceedings*

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ISOLATION AND CHEMICAL CHARACTERIZATION OF HEMICELLULOSES FROM RYE BRAN

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ABSTRACT: Several methods have been suggested for hemicellulose isolation from cereals and cereal brans and extraction processes for water soluble hemicelluloses have been developed for soluble dietary fibers in the food industry. Using these methods, water soluble hemicelluloses can be readily extracted as high molecular weight polymers and the relevance of these materials can be evaluated in non-food applications. Water-soluble hemicelluloses of rye bran were extracted with a high-temperature treatment combined with enzymatic starch removal. After the hot water extraction, non-soluble fibers and protein fractions were separated and the washed fiber fraction was further treated with alkali (NaOH) solution with different solid to liquid ratios. The ratio of arabinoxylans (~65%) and β -glucans (~25%) were similar in the water-extracted and alkali-extracted materials, however their ara/xyl ratio differed. The alkali-extracted arabinoxylan was less substituted with an ara/xyl ratio of 0.35, while the water-extracted material had an ara/xyl ratio of 0.54. High molar mass hemicellulose materials were isolated with an average molecular weight of 700 000 g/mol with both isolation processes.

Keywords: hemicelluloses, agricultural residue, isolation

1 INTRODUCTION

Cereal brans such as those extracted from wheat and rye are by-products of the conventional milling process. They are mainly used as animal feed [1] although many industrial applications can be found such as in viscosity modifiers, gelling agents or tablet binders [2]. Furthermore, there is an increasing interest in using hemicellulose-rich dietary fibers from cereal brans for human consumption. Arabinoxylans and mixed linkage β -(1 \rightarrow 3, 1 \rightarrow 4)-D-glucans (henceforward denoted as β -glucans) are constituents of cereal dietary fibers and have been studied because of their medicinal effects. They can reduce the occurrence of diabetes, cardiovascular diseases or colon cancer and also reduce blood cholesterol and glucose levels [2-4]. Xylans show excellent emulsifying properties and can be applied as protein foam stabilizers [5]. Considering the molecular and physicochemical properties of these molecules, there is considerable potential for formation of value-added materials. The hemicellulose-rich cereal brans are potential sources for biopolymers due to their arabinoxylan content.

The bran forms the outer parts of the grains including several layers of the grain coat (e.g. the pericarp, cuticle, the testa (or the seed coat) and the aleurone layer). Commercial bran preparations also contain variable amounts of the starchy endosperm and germ depending on the milling process [6, 7]. The total arabinoxylan content of the bran is usually higher than that in the endosperm and therefore milling leads to different fractions with different hemicellulose contents. Although more arabinoxylan can be found in the bran, the amount of water-extractable arabinoxylan is higher in the endosperm-rich fractions [7].

Rye bran hemicelluloses are mostly arabinoxylans and β -glucans to a minor extent and both are embedded and bound with other components in the secondary cell walls. Rye water-extractable arabinoxylans typically have a chain of (1 \rightarrow 4)-linked β -D-xylopyranose units containing L-arabinofuranose residues connected principally to the C3 or C2 and C3 positions [7]. The arabinose units can be ester-linked to ferulic acid residues which may form diferulic acid bridges neighboring with arabinoxylan chains [8]. Arabinoxylans can probably also be covalently associated with proteins through ester-

linked ferulic acids and tyrosine residues [8, 9]. In cereal grain cell walls, the arabinoxylan is closely associated with mixed linkage β -glucans as well, especially in the endosperm part [10].

Extraction of hemicelluloses from the cell walls is based on their solubility and can be carried out in neutral or alkaline solutions [11]. Hence, hemicelluloses are divided into two fractions: water-soluble and water-insoluble [12]. Difficulties in carrying out water extraction of cereal bran xylans may arise because hemicelluloses is bound to lignin or cellulose through ferulic acid bridges and also because of hydrogen bonding between the non-substituted xylose residues and the cellulose chains [7, 13, 14].

Several processes have been introduced for hemicellulose isolation from grain crops and from cereal brans, involving water and alkali extraction as well as other combinations such as alkali and hydrogen peroxide, alkali and chlorite solutions or dimethyl sulfoxide [15]. In addition, pilot-scale isolation of cereal xylans has been demonstrated, indicating the feasibility of scaling up to an industrial level [16-19].

Only 20-40% (w/w) of cereal grain hemicelluloses is typically water-extractable [7, 20, 21]. Water extraction allows the isolation of high molar mass hemicelluloses and helps preserve the hemicellulose structure although the resulting yields are relatively low [15]. A general method has been demonstrated for water extraction by Bengtsson and Åman [22]. Yields can be highly improved by extraction with other solvents, most commonly applied under alkaline conditions. Such treatments can cause deacetylation in the case of certain hemicelluloses so the original structure will not then be preserved. Selective arabinoxylan extraction, avoiding the co-isolation of β -glucan, can be performed with barium hydroxide solution contrary to sodium or potassium hydroxide solutions [14, 23]. Separation of arabinoxylans and β -glucans can also be performed via precipitation with saturated $(\text{NH}_4)_2\text{SO}_4$ or through enzymatic digestion [24, 25].

Alkaline extractions are often associated with lignin removal using sodium hypochlorite, chlorine or hydrogen peroxide treatments [13, 26]. Higher yields can be obtained from lignified materials using dimethyl sulfoxide as a delignifying agent but the use of this solvent is not applicable in pilot scale or industrial

isolation processes [15]. As a consequence, a range of multi-step extraction processes have been proposed for such polysaccharide isolations [14, 23, 25, 27-30].

Additional enzyme treatments are usually necessary to obtain a high-purity hemicellulose extract but the presence of components such as starch and proteins can in addition hinder the isolation of xylans [31, 32]. Amylase enzymes, such as α -amylase and amyloglucosidase are applied for starch degradation and protein removal is generally carried out with protease enzymes [33]. Additional treatments like ultrasonication can be of benefit by providing separation of co-extracted starch and protein from the isolated hemicelluloses. Hollmann et al. showed that ultrasonication reduced the extraction time of alkali-treated arabinoxylans from wheat bran [26].

The present study was aimed at isolation of high molar mass hemicelluloses from rye bran. These hemicelluloses might then be used to produce industrially useful biodegradable materials. The extractability, chemical composition and structure of the water-extractable hemicelluloses was examined based on a hot water isolation process, while the residual water-insoluble material was subjected to an alkaline treatment. The molar mass distribution of the isolated hemicelluloses and the effect of isolation method on hemicellulose structure were studied.

2 EXPERIMENTAL

2.1 Materials

Rye (*Secale cereale*) cultivar Carotop was grown in Denmark in 2008. Rye grains were disc milled and the fine flour fraction was separated. Material with particle size in the range 0.25-1.0 mm and with a mean diameter of 0.5 mm was used for hemicellulose extraction. The bran fraction was analyzed in accordance with the procedures described below.

Thermostable α -amylase Termamyl SC was obtained from Novozymes A/S (Bagsvaerd, Denmark). Amyloglucosidase (EC 3.2.1.3 from *A. niger*) was purchased from Megazyme International Ireland Ltd. (Bray, Ireland).

2.2 Composition of rye bran

Total sugar composition was determined by HPLC analysis after sulphuric acid hydrolysis. In this procedure, 1.5 ml of 72% H_2SO_4 was added to 0.16 g sample and pre-hydrolyzed for 60 minutes at 30 °C. After dilution with Millipore water (42 ml), samples were autoclaved at 120°C for 60 minutes. The filtered liquid was analyzed on an HPLC column (Aminex HPX-87H, Bio-Rad, Hercules, CA, USA), while the residue was heated to 550 °C to determine the lignin ash content [34, 35]. Glucan, xylan and arabinan content were determined from the liquid phase. For HPLC analysis, 40 μl samples were injected at a temperature of 63°C and flow rate of 0.6 ml/min (eluent 4 mM H_2SO_4). Analytes were detected by a refractive index (RI) detector (Shimadzu, Japan). Samples were analyzed in duplicates.

Starch content was determined using a Laboratory Analytical Procedure (LAP) of NREL (Issue Date: 07/17/2005). In this case, 100 mg of milled sample and starch standard was weighed and 0.2 ml ethanol was added to aid sample dispersion. Subsequently, 2 ml of dimethyl sulfoxide (DMSO) was added and the tubes

were placed in a briskly boiling water bath for 5 minutes. Then, 2.9 ml MOPS buffer and 0.1 ml thermostable α -amylase were added, mixed and the mixture was incubated in a boiling water bath for 6 minutes, with stirring every two minutes. Tubes were placed in a 50°C water bath and 4 ml sodium acetate buffer (pH 4.5), followed by 0.1 ml amyloglucosidase enzyme, was added and mixed well. After incubating for 30 minutes at 50°C samples were removed and centrifuged for 10 minutes at 2000 g. Samples for glucose determination were analyzed in triplicate by HPLC.

Soxhlet extraction of rye bran was carried out for 24 hours using 96% (v/v) ethanol in order to determine the lipid content according to the ASTM Standard E1690, 2008 [36].

The ash content was measured gravimetrically after ashing at 550°C.

The protein content of rye bran was calculated from the total amount of measured nitrogen in the samples ($5.83 \times \text{N}$). The total nitrogen content was determined with an EA 1110 CHNS-O elemental analyzer (CE Instruments, Wigan, UK) at an 1800°C combustion temperature.

Mixed linkage β -glucan was analyzed by the method of McCleary and Glennie-Holmes (ICC Standard Method No. 168) using a Megazyme assay kit (Megazyme International, Bray, Ireland) [37]. Samples were analyzed in triplicate.

2.3 Isolation of rye bran water-extractable hemicelluloses

The isolation processes are illustrated in Figure 1. Rye bran slurry (bran to water ratio = 1:7 w/v) was treated with Termamyl SC at pH 6.0 with continuous stirring. After addition of α -amylase (dosage: 0.2 w/v% of residual starch mass), starch was gelatinized for 45 minutes at 95°C. Fragmentation of particles was carried out using a wet mill (Mannesmann, Remscheid, Germany) during the extraction procedure. Water-insoluble material (WIS), bran fibers, proteins and waxes were separated from the supernatant syrup by centrifugation (approx. 6000 g for 15 minutes). The syrup (water soluble – WS) fraction was treated again with Termamyl SC for 45 minutes at 95°C. Treated sugar syrup was autoclaved for 5 minutes at 120°C and α -amylase was deactivated. Further separation of precipitated proteins was performed by centrifugation (approx. 6000 g, 10 minutes). The pH of the samples was reduced to 4.5 with 5 M HCl. Enzymatic digestion of glucose oligomers was performed with amyloglucosidase (dosage: 0.2 w/v% of residual starch mass) at 60°C for 45 minutes. Sugar syrup was dialyzed (MWCO 12000-14000 Da) at room temperature for 24 hours against water to remove the glucose and oligomer units originating from starch. The aqueous extract was collected and precipitated with an equal volume of ethanol (96% v/v) and left overnight at 4°C. The precipitate was collected after centrifugation (approx. 6000 g, 20 minutes). Hemicellulose gum was washed with a 1:1 (v/v) mixture of ethanol and water. The gum was then resuspended in distilled water and freeze dried. The fiber fraction of the separated WIS material was extracted with NaOH according to Ragaei et al [24]. The bran fibers were washed with 500 ml, centrifuged for 15 minutes at approx. 6000 g and dried at 45°C overnight. NaOH extraction was performed with 1 M NaOH at 25°C for 2 hours with continuous magnetic stirring at fiber to liquid ratios of: 1:10; 1:35; 1:70. The mixture was

neutralized with 5 M HCl after the treatment and centrifuged for 20 minutes at approx. 4000 g. The hemicellulose-containing supernatant was dialyzed (MWCO 12000-14000 Da) for 24 hours at room temperature against deionized water and hemicelluloses were precipitated with ethanol then freeze dried as described above.

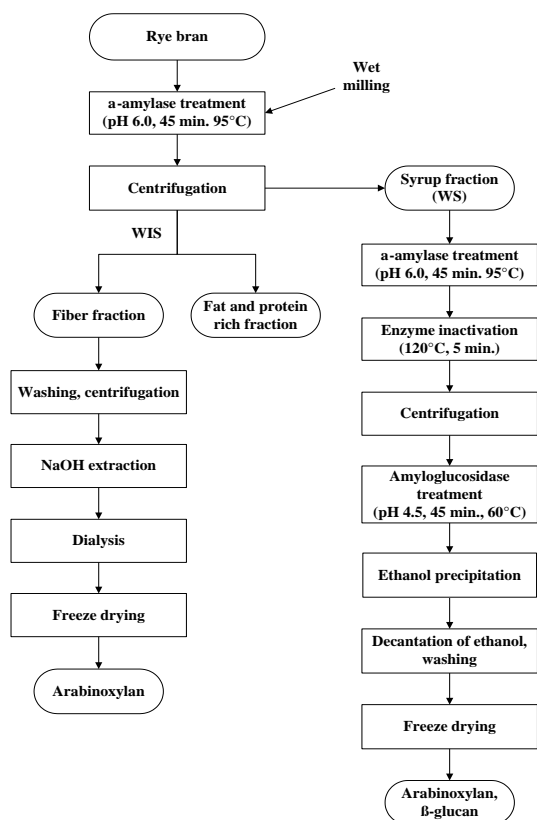


Figure 1: Hemicellulose isolation procedure starting from rye bran

2.4 Sugar composition of the isolated material

Monosaccharide composition was determined by HPLC analysis. Before analysis, samples were treated with 4% (w/v) sulphuric acid and autoclaved for 10 minutes at 121 °C. Samples were neutralized with CaCO₃ and filtered (45 µm) for HPLC analysis. Samples were analyzed in duplicate.

In the HPLC analysis, 40 µl samples were injected at a temperature of 85°C and flow rate of 0.6 ml/min (eluent Millipore water) on an Aminex HPX-87P (Bio-Rad, Hercules, CA, USA) column. Analytes were detected using an RI detector (Shimadzu, Japan).

2.5 Gas chromatography-mass spectrometry (GC-MS) analysis of methylated and acetylated sugars

Sugar compositional analysis was performed using gas chromatography-mass spectrometry analysis after acid methanolysis and acetylation of sugar samples. Hemicellulosic samples (approx. 10 mg) were degraded to monosaccharides using 5 ml 1.25 M HCl in methanol (Sigma Aldrich, Germany) [38]. The samples were kept at 100°C overnight and neutralized with CaCO₃ (approx. 0.5 g). Following this step, samples were filtered and washed with 5 ml methanol. After evaporation of methanol, samples were dissolved in 4 ml of an acetic anhydride-pyridine mixture (1:4) and heated at 100°C for

30 minutes. After cooling to ambient temperature, 20 ml CHCl₃ was added to the mixture. The samples were washed as follows: 25 ml Millipore water, 25 ml 2M HCl, 25 ml Millipore water, 25 ml 5 % NaHCO₃, 25 ml Millipore water, while the organic phase was isolated and then dried with anhydrous Na₂SO₄.

Monosaccharide standards (D-glucose, D-xylose, D-arabinose) and acids (D-galacturonic acid, D-glucuronic acid) were treated with the above described method as well. The derivatized sugars were analyzed by GC-MS using a Hewlett Packard HP 6890 gas chromatograph interfaced to a HP5973 Mass Selective Detector (Agilent, Denmark). A sample of 1 µl was injected using an HP 7683 auto sampler (Agilent, Denmark) and introduced in a split mode (1:20). The source and rod temperature were 230 °C and 150 °C respectively. The products were separated using a 0.32 mm i.d. x 30 m WCOT fused silica column coated with VF-23ms at a thickness of 0.25 µm (Analytical, Denmark). The carrier gas was He at a flow rate of 1.2 ml/min. Separation of a wide range of products was achieved using a temperature program from 70 °C to 250 °C. Full mass spectra were recorded every 0.3 s (mass range m/z 40 – m/z 450). Products were identified using NIST search engine, version 2.0 f. (Agilent, Denmark).

2.6 Size exclusion chromatography analysis

Molar mass determinations were carried out using size exclusion chromatography (SEC). Samples were dissolved in 1M NaOH (4 mg/ml) by stirring overnight, diluted four times in the eluent (0.01 M NaOH, 50 mM NaCl, pH 12) and filtered using 0.45 µm syringe filters (PTFE) before analysis. Samples (200 µl) were injected on a TSK-Gel G4000PW column (7.5 x 600 mm, ToSoHaas, King of Prussia, USA) with a TSK-Gel G2500PW guard column (7.5 x 600 mm). The eluent flow rate was 0.5 ml/min. Three detectors were used to monitor the resulting peaks: a light scattering detector (Model 270 dual detector, Viscotek Corp.), a differential refractometric detector (Shimadzu) and a UV-VIS photodiode array detector (Shimadzu). Conventional calculations were made using TriSEC 3.0 software (Viscotek Corp.). Data were referred to pullulan standards in the molar mass range of 5600-1.6 mill g/mol.

3 RESULTS AND DISCUSSION

3.1 Composition of rye bran

The composition of rye bran is shown in Table 1. The high starch content of ~50% w/w should be noted. This amount is in contrast with the starch content of cereal brans provided by industrial mills, which typically vary in the range of 13-28% w/w [6, 14, 30]. The grains were processed using a disc mill instead of industrial roller milling which may explain this difference starch content and probably reduced the hemicellulose content in the raw material [4, 6]. The disc milling supposedly provided a slightly different bran structure with a higher amount of starch granules originating from the endosperm. Starch molecules are attached to the aleurone layer of the grains and were separated by the milling. The starchy endosperm particles likely originated mainly from the outer parts of the endosperm, the subaleurone and prismatic cells, since the inner endosperm parts were separated and recovered as fine rye flour [31]. As a result, the pentosan content of the bran material was lower (~13% w/w) when disc milling was used. The non-

starch glucan was comprised mainly of β -glucan (2.8% w/w) and also cellulose. Nilsson et al. reported a higher amount of β -glucans (3.4%) which was nevertheless in a very similar range. Rakha et al. found 4.4% β -glucan, while Kamal-Eldin et al. measured 5.3% in a rye bran from Finland [4, 6, 14]. The cellulose and Klason lignin contents were in accordance with the results given for rye bran in previous reports [6]. The ash content of the material was lower than previously measured values (2.8-6.5% w/w) as was the measured protein content [6, 31]. Minerals are in general concentrated in the bran fraction with the lowest mineral content in the endosperm part of the grains. Compositional differences therefore occurred due to the different milling procedures, which will influence the amount of endosperm particles in the material.

Table 1: Composition of rye bran

Component	g/100 g dry material
Xylan	8.6 \pm 0.2
Arabinan	4.0 \pm 0.1
Glucan (starch)	49.6 \pm 2.9
Glucan (non-starch)	10.6
β -glucan	2.8 \pm 0.1
Klason lignin	4.6 \pm 0.2
Protein	10.8 \pm 0.1
Extractives	9.9 \pm 0.2
Ash	1.4 \pm 0.1

3.2 Water-extractable hemicelluloses

The isolation procedure shown in Figure 1 using a high temperature treatment allowed the recovery of water-extractable hemicelluloses. The wet milling had the effect on making the starch granules more available for the amylase enzyme when compared with previous extractions, starch granules were more available to the α -amylase and the final glucose content decreased by approx. 20% (data not shown). Fibers, proteins and waxes were separated after the high temperature treatment; however, additional protein separation was necessary. Remaining proteins were precipitated during the autoclave treatment and separated by centrifugation. Further purification of the isolated hemicelluloses was needed and consisted primarily of the removal of starch residuals. After amyloglucosidase enzyme treatment, dialysis was performed. Dialysis was applied in order to remove mono- and oligosaccharides as well as buffer ions, peptides and proteins which were smaller than the membrane cut-off value (12000-14000 Da). Fractional precipitation of the isolated hemicelluloses was not applied to avoid further loss of the material and decreasing yields. Delcour et al. applied a clay treatment in order to reach further purified material with protein separation which left no residual proteins in the arabinoxylan material [19]. With the applied method, a 93.4% pure arabinoxylan was isolated which is a higher purity than described earlier by Bengtsson and Åman (72%) [22]. Faurot et al. applied a heat treatment to precipitate water-soluble proteins from the supernatant phase after water extraction from wheat flour, which treatment led to relatively high protein content in the pentosan extracts, varying from 30% to 50% w/w [18]. The high protein contents could be further decreased with

proteinas treatment since the precipitation and separation by centrifugation resulted in significant residual protein amounts.

Water-extracted arabinoxylans represented 25% of the total arabinoxylan content of the bran which showed an efficient extraction yield considering the low amount of water-extractable hemicelluloses. Ragae et al found that 22 to 33% of the total arabinoxylan content of different rye meals was water extractable [39]. However extraction yield calculated from the starting bran material was rather low, since the isolated material was only 2.7% w/w of the starting bran. The losses during the fraction separations, dialysis and precipitation further decreased the yield. Cyran et al. reached slightly higher yields, approximately 4% [31]. The resulting sugar composition (Table 2) showed a major amount of xylose and arabinose and a significant amount of glucose monomers. Presumably, the dialysis process removed most of the degraded starch molecules and the extraction method allowed the co-isolation of β -glucans. The resulting 0.54 ara/xyl ratio was in agreement with other water-extracted arabinoxylans isolated from rye [19, 22, 39]. The high protein content might be a consequence of existing covalent linkages between arabinoxylan chains and proteins. Ragae et al. found that water-extracted arabinoxylan contained 3-5% (w/w) proteins even after enzymatic digestion [39], while Cyran et al found the fraction after proteinase digestion to be enriched with 61-65% proteins [31]. The specific presence and composition of aromatic constituents could have blocked the enzymatic action and this would be consistent with an association between the polysaccharides and proteins in the cereal cell walls.

Table 2: Composition of water-extracted hemicelluloses

Component	g/100 g dry material
Total monosaccharides	92.1
Arabinose	22.5
Xylose	41.7
Glucose	23.6
Galactose	1.6
Fructose	2.8
ara/xyl	0.54
Protein	11.4
Yield ^a	3.0

a: Expressed as weight percentage of rye bran

3.3 Alkaline-extractable hemicelluloses

After the water extraction process, the amount of water-insoluble material was approx. 40% (w/w), which consisted of a fraction rich in proteins and waxes and a fiber fraction. Nilsson et al. isolated polysaccharides from 3 milling fractions of rye, a bran, an intermediate and a flour fraction [14]. After water extraction, the remaining WIS material constituted 50% of the starting bran and 25% for the intermediate fraction and found a starch content of less than 2% in these fractions.

The WIS fiber fraction was separated (Figure 1) and the composition was analyzed and is shown in Table 3. The obtained fiber fraction made up 27% of the starting rye bran. This separated amount of fibers was in good agreement with previously found yields after extraction

with α -amylase and proteinase enzyme treatment on rye grain outer layers [40]. The major building components of the WIS fibers were polysaccharides. Almost 30% w/w of the fiber fraction was arabinoxylan; however there was also a large amount of Klason lignin and a high proportion of proteins present in the fraction. A fairly high percentage of the measured glucose residues originated from cellulose and starch residues since the β -glucan content was only approx. 15% (w/w) of the total glucose content. The washing process decreased the amount of glucose residues by 34%, proving that a significant amount originated from starch degradation. The protein content was ~13% and higher than that previously measured in the study by Cyran et al [40].

Table 3: Composition of the WIS material (before alkaline treatment)

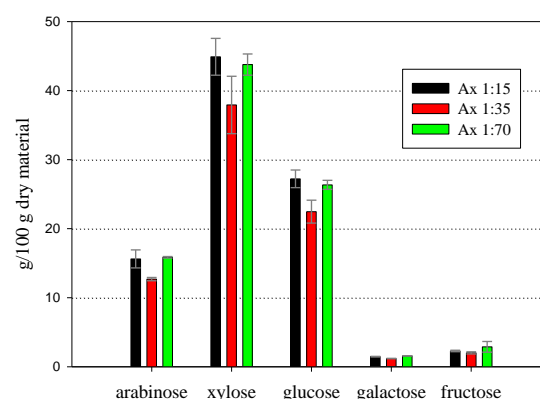
Component	g/100 g dry material
Protein	18.4
β -glucan	4.7
Total monosaccharide	60.9
Arabinose	9.1
Xylose	18.4
Glucose	33.4
Klason lignin	10.3

Different ratios of the washed rye fiber and 1 M NaOH solutions were mixed to see the effect of the solid to liquid ratio on the isolation efficiency. In order to separate the resulted small molecules, dialysis was used. Figure 2 shows the sugar components of the NaOH extracted fiber material. The ara/xyl ratios (data not shown) were in the same range in all cases (0.35, 0.34 and 0.36 respectively), which suggested that the xylan chain was less substituted than in case of the water-extracted arabinoxylan. The decrease in branching was observed in previous studies in the outer layers of the rye grains compared to an intermediate milling fraction or a whole flour [14]. The monosaccharide composition of the Ax 1:15 and Ax 1:70 were almost identical while the Ax 1:35 showed slightly lower results from all the sugars. However the overall yield was the highest for the isolated arabinoxylan Ax 1:35 material, giving 66% of the total arabinoxylan content of the washed fiber material while only 41% and 45% were the results with the materials Ax 1:15 and Ax 1:70 respectively (data not shown). Cyran et al used 1 M NaOH and even stronger 4 M NaOH solution for arabinoxylan extraction and found that some arabinoxylan structures were closely associated with cellulose and therefore the use of stronger alkaline solutions than 1 M NaOH was necessary [40]. Ragaee et al. showed that higher concentrations of NaOH could dissolve more β -glucans [41]; however, this also induced depolymerization of the polysaccharides. The polysaccharide content of the isolated materials for Ax 1:15, Ax 1:35 and Ax 1:70 was 91%, 76% and 90% respectively, suggesting the presence of smaller amounts of other components like proteins and Klason lignin [41]. The presence of Klason lignin was indicated as well by the material's darker color. Cyran et al found that rye arabinoxylans were associated with proteins and lignin components and these were present in 1 M NaOH

extracts even after water extraction with α -amylase and proteinase [40].

Considering the amount of chemicals needed, the 1:35 treatment would be the most beneficial solution for such extractions, since this experiment showed the highest hemicellulose yield.

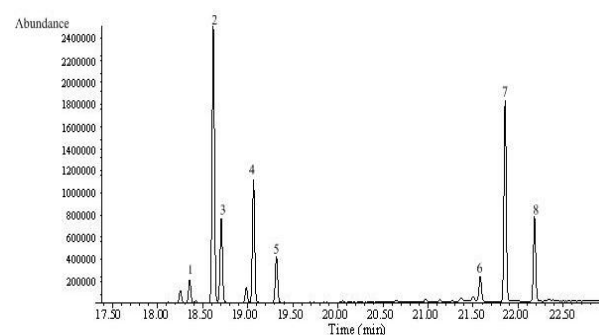
Figure 2: Monosaccharide composition of NaOH extracted rye fibers with solid to liquid ratios: 1:15, 1:35, 1:70



3.4 Monosaccharide analysis of isolated hemicelluloses

A more thorough investigation of the monomer composition of the water-extracted material was performed by acid methanolysis, sugar derivatization and GC-MS detection. Derivatization involved peracetylation using acetic anhydride. Acetylation was applied as derivatization for the methyl glycosides although the most common method is per(trimethylsilyl)ation. Contrary to silylation, the prepared acetates were very stable so they could provide more information for a longer period than silylated products and might be used for further branching studies as well.

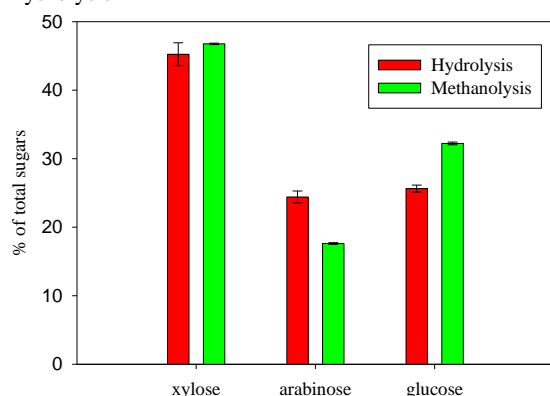
Figure 3: GC-MS chromatogram of isolated hemicellulosic material after acid methanolysis and acetylation. Peaks 1-5 represent xylose and arabinose, peaks 6-8 represent glucose residues.



The analysis method has the potential to be more suitable for hemicellulose analysis than acid hydrolysis since the sugar acids are protected and detectable after chemical modification. The obtained chromatogram can be seen in Figure 3. Peaks 1-5 show arabinose and xylose while peaks 7-9 are hexose sugars, including glucose. Glucose suffered degradation during the analysis; however, the ratio of the degradation products remained

the same in several repeat analyses. Sugar identification and quantification was done by analysis based on the peak retentions and peak areas compared with previously analyzed mono- and polysaccharide standards. The drawback of this analysis is that during sample preparation, a mixture of α - and β -anomers as well as pyranose and furanose forms were obtained, hence no information on such structures could be obtained since up to four isomers of one single sugar unit could be identified [42].

Figure 4: Monomer composition, expressed as % of the total sugar amount with acid methanolysis and acid hydrolysis



The wet chemical analysis involving use of dilute sulphuric acid and HPLC analysis showed the presence of glucose, xylose, and arabinose as major compounds as well as the presence of fructose (Table 2). The relative sugar composition was calculated and the comparison of results based on acid methanolysis and hydrolysis is shown in Figure 4. Comparing the two sugar analysis procedures a higher xylose and glucose content could be found with acid methanolysis. The arabinose level was lower in the case of methanolysis and the xylose amount was very similar which could result from incomplete degradation of the arabinoxylan structure in the case of acid methanolysis. Sundberg et al. compared acid methanolysis and acid hydrolysis results for wood hemicelluloses and found a higher xylose, mannose and glucose content when using hydrolysis, assuming that cellulose glucose units were also cleaved [38]. It was shown that most of the glucose units formed by methanolysis originate from non-cellulosic components as the method does not degrade crystalline cellulose. This is in contrast with results from acid hydrolysis. Willför et al. compared different carbohydrate analysis methods performed in different laboratories and found that methanolysis was a more suitable method for xylan and uronic acid-containing sample analyses, in which labile sugars were not degraded as they are during acid hydrolysis [43]. However, acid methanolysis enables analysis of both neutral and acidic carbohydrates in one run and provides excellent separation of the obtained sugars. Although methanolysis data are more reproducible, a longer sample preparation time is needed. Further, the separation capability and sensitivity of the GC-MS system is higher than that of the HPLC system. Acid methanolysis has been shown to be a suitable method for isolated hemicellulose analysis and has been applied to different raw materials such as wood, pulp fibres, or wheat straw samples. (Sundberg, 1996, Bertaud, 2002, Willför, 2009, Virkki, 2008, Pitkanen, 2008).

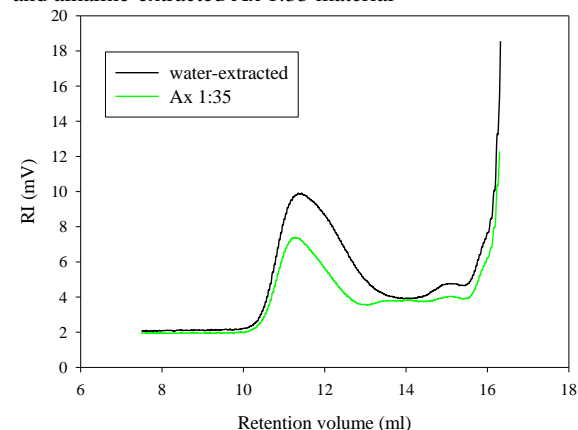
3.5 SEC - molar mass distribution

Molar mass analysis of the isolated hemicelluloses was analyzed by SEC. Conventional calibration was used for molar mass calculations based on the response of a range of pullulan standards. Hemicellulosic materials tend to form aggregates in solution. Since this behavior likely occurs during the analysis, the light scattering signal may lead to false molar mass calculations.

The obtained chromatogram indicated that high molar mass materials were isolated in both cases, using the water extraction and the alkaline treatment. Figure 5 shows the refractive index (RI) signals of the water-extracted and the Ax 1:35 alkali-extracted materials. The chromatograms showed a slightly higher hydrodynamic volume for the water-extracted material. The calculated average molar masses of the isolated hemicelluloses were in a similar range ($M_w = 729\,900$ g/mol for the water-extracted and $M_w = 744\,600$ g/mol for the Ax 1:35 material). A smaller amount of low molecular weight components could be seen, although those signals might be partly covered by the unbalanced signal of the eluent. Rather wide peaks are observable especially in case of the water-extracted material, indicating a mixture of molecules having a wide range of different molar masses. Such behavior was observed previously by Cyran et al. for water-extracted cereal arabinoxylans [31]. Additionally the high polydispersity of the two studied hemicellulosic materials refers to a wide range of molar masses ($Pd = 6.84$ and 4.83 for water-extracted material).

Molar mass distribution of isolated cereal hemicelluloses has been thoroughly investigated. The calculated molar mass often depends on the SEC system, the eluent and the calculation method, so the measured average molar mass can vary between 2×10^4 and 9×10^5 g/mol [31, 44]. Pitkanen et al. found a lower weight average molecular weight ($246\,400$ g/mol) of water-extracted rye arabinoxylan which was dissolved in DMSO [44], while Cyran et al. found fractions of water-extracted rye hemicelluloses with 9.34×10^5 and 5.49×10^5 g/mol dissolved in a NaNO_3 solution [31].

Figure 5: SEC profiles of water-extracted hemicelluloses and alkaline-extracted Ax 1:35 material



4 CONCLUSIONS

Hemicellulose extractions from rye bran were performed using hot water and alkaline treatments. The original rye bran material was rich in starch, containing a

fairly high amount of the endosperm part of the grains. The hot water-extracted material was treated with starch degrading enzymes followed by dialysis and the resulting material contained mainly arabinoxylan (~65%) and co-extracted β -glucans (~20%). The remaining water-unextractable material was alkali-extracted and this resulted in a material with a similar content of arabinoxylan and β -glucan as the water-extracted material. The alkali-extracted material had a lower arabinose substitution with a lower ara/xyl ratio (0.35) than the water-extracted material (0.54). Acid methanolysis was proven to be a suitable method for monosaccharide analysis. Acid methanolysis resulted in a slightly different monosaccharide composition than acid hydrolysis. It showed a higher xylose and glucose content and a lower arabinose level. Methanolysis data could be more reproducible and the sensitivity of the GC-MS system is higher than that of the HPLC system to detect the chemically modified monomers. High molecular weight materials (~700 000 g/mol) were isolated; the extraction procedure did not have a significant effect on the molecular weight. The polydispersity of the two types of hemicelluloses however showed some differences (Pd=6.84 for alkali-extracted and 4.83 for water-extracted material). High molar mass hemicelluloses can be isolated with a similar structure using a hot water or an alkaline treatment however the extraction yields are higher with alkali extractions. The choice of extraction processes should be dependent on the application purposes for the hemicellulosic materials.

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6 ACKNOWLEDGEMENTS

Anne Belinda Thomsen and Mark Lawther are acknowledged for supervising the extraction processes. We are grateful to Ingelis Larsen and Lotte Nielsen for technical assistance.